

## Isotopic composition of maize as related to N-fertilization and irrigation in the Mediterranean region

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**ABSTRACT:** Nitrate leaching as a result of excessive application of N-fertilizers and water use is a major problem of vulnerable regions. The farming of maize requires high N fertilization and water inputs in Spain. Isotopic techniques may provide information on the processes involved in the N and C cycles in farmed areas. The aim of this work was studying the impact of sprinkler and furrow irrigation and N input on maize (*Zea mays* L.) yields, and whether isotopic composition can be used as indicator of best farming practices. Trials were set up in Tudela (Spain) with three rates of N fertilization (0, 240 and 320 kg urea-N ha<sup>-1</sup>) and two irrigation systems (furrow and sprinkler). Yield, nitrogen content, irrigation parameters, N fate and C and N isotope composition were determined. The rate of N fertilization required to obtain the same yield is considerably higher under furrow irrigation, since the crop has less N at its disposal in furrow irrigation as a result of higher loss of nitrogen by NO<sub>3</sub><sup>-</sup>-N leaching and denitrification. A lower δ<sup>13</sup>C in plants under furrow irrigation was recorded. The δ<sup>15</sup>N value of plant increased with the application rate of N under furrow irrigation.

**Key words:** *Zea mays* L., furrow irrigation, nitrogen, sprinkler irrigation

## Composição isotópica do milho relacionada à fertilização nitrogenada e método de irrigação na região do Mediterrâneo

**RESUMO:** A lixiviação de nitratos resultante da aplicação excessiva de fertilizantes nitrogenados e o uso excessivo de água são problemas sérios em regiões vulneráveis. A cultura do milho (*Zea mays* L.) na Espanha exige altos níveis de fertilização nitrogenada e irrigação. Técnicas isotópicas podem prover informações sobre os processos envolvidos nos ciclos do N e do C em área agrícolas. Avaliou-se o impacto da irrigação por aspersão ou sulco e adição de N na produtividade do milho e se a composição isotópica pode ser usada como indicador de melhores práticas de manejo da produção agrícola. Os ensaios foram realizados em Tudela, Espanha, utilizando três níveis de fertilização nitrogenada (0, 240 e 320 kg ureia-N ha<sup>-1</sup>) e dois sistemas de irrigação (sulco e aspersão). Foram determinados produtividade, teor de nitrogênio, parâmetros de irrigação, fixação de N e C e composição isotópica dos grãos. Os níveis de N exigidos para obtenção de produtividades idênticas são maiores sob a irrigação em sulcos, uma vez que nestas condições as perdas de nitrogênio pela lixiviação de NO<sub>3</sub><sup>-</sup>-N e denitrificação são maiores e, consequentemente, a disponibilidade de N é menor. Foi registrado menor δ<sup>13</sup>C nas plantas irrigadas por sulcos. Os valores do δ<sup>15</sup>N nas plantas irrigadas por sulcos aumentaram com os níveis de fertilização nitrogenada.

**Palavras chave:** *Zea mays* L., irrigação por sulco, nitrogênio, irrigação por aspersão

### Introduction

According to the European directive on nitrate-vulnerable zones (91/676/EEC), the improved management of N nutrition of irrigated crops is instantly required. Maize (*Zea mays* L.) crops in nitrate-vulnerable Mediterranean zones require much higher input of mineral N as a result of prevalent farming of the crop and its high N requirements. The water-limited conditions of the Mediterranean climate make irrigation an absolute need for better crop yields (Di Paolo and Rinaldi, 2008). Therefore, improvement of the quality of aquifers depends on quality management of maize crops. Numerous authors have investigated the effect of N and water management on the efficiency parameters of maize (Dagdelen et al., 2006; Di

Paolo and Rinaldi, 2008) but most of these studies compare efficient irrigation with deficit irrigation systems, instead of comparing different irrigation systems.

The natural abundance of C and N isotopes in higher plants can be used to compare their physiology and environmental effects. Probably because of the higher complexity of the N cycle, the natural abundance of N isotopes (<sup>15</sup>N/<sup>14</sup>N ratio) has been used than less intensively that of C isotopes (<sup>13</sup>C/<sup>12</sup>C ratio) in plant physiology and ecology studies (Farquhar et al., 1989; Höglberg, 1997). Carbon isotope discrimination is a good measure of leaf transpiration efficiency in C<sub>3</sub> plants and has been proposed as a select criterion for greater water efficiency in breeding programs in water-limited environments (Farquhar and Richards, 1984; Condon et al., 1992).

However, the relationship between carbon isotope discrimination and drought effects in  $C_4$  plants is still controversial (Monneveux et al., 2007).

Few studies and with contradictory results are found regarding how N fertilization affects  $\delta^{13}C$  of plant (McDonald and Davies, 1996; Shangguan et al., 2000, the emphasis been put more frequently on the comparison of N sources (Lopes and Araus, 2005). On the other hand, several authors have shown that N cycle processes can change the  $^{15}N$  isotope composition of the soil and, therefore, of the crop (Mariotti et al., 1981; Högberg, 1997; Choi et al., 2001).

The aim of this investigation was to evaluate the environmental effect of two irrigation systems, furrow and sprinkler, and N application rate on the maize yield. It also intended to test the suitability of  $\delta^{13}C$  and  $\delta^{15}N$  as indicators for improving good agricultural practice in the management of irrigation and nitrogen fertilization.

## Material and Methods

The field study with maize (var. DRAGMA) was conducted in an irrigated area in Tudela, Spain ( $42^{\circ}5'N$ ,  $1^{\circ}36'W$ ), in a Xerollic Paleorthid soil, in a 1-m deep alluvial terrace with sandy loam texture, pH 8.5, organic carbon content of 0.8 %, and a  $\delta^{15}N$  of 6.7. Area's climate is steppe semi-arid (av O) according to Papadakis Climate Classification (Papadakis, 1960). The maize crop was sown at 72 cm between rows and 18 cm between plants. Phosphorus ( $P_2O_5$ ; 140 kg ha $^{-1}$ ) and potassium ( $K_2O$ ; 180 kg ha $^{-1}$ ) were added at sowing in all plots. Two adjacent plots with two types of irrigation (furrow and sprinkler) were treated with rates of N in a completely randomized block design (n=4). The size of each micro plot was 10 × 6 m. Nitrogen was applied in one side dress in V6 phenological phase and treatments were: 0 (control), 240 (dose recommended in the zone) and 320 kg of N ha $^{-1}$  (over-fertilization dose). Nitrogen (urea) was added manually to the plots and incorporated into the soil during the first irrigation cycle. Sprinkler irrigation was done every two days according to the recommendations of Allen et al. (1998) (total irrigation cycles: 30; average quantity per cycle: 20 L m $^{-2}$ ) and furrow irrigation was done once a week, mimicking the procedure of most maize producers in the area, with a total of 10 irrigation cycles (average quantity per cycle: 108 L m $^{-2}$ ). Leaching percentage, expressed as drainage water between total amount of irrigation water plus rain water, and irrigation water use efficiency (IWUE), determined as yield (kg ha $^{-1}$ ) per unit irrigation water applied (mm) were determined to estimate the efficiency of the two irrigation methods (Howell et al., 1990).

Nitrogen fate was determined at 0-120 cm depths. N inputs (kg N ha $^{-1}$ ) were estimated as the sum of soil mineral N at pre-sowing, N applied as fertilizer in the side dress, N applied with irrigation water, and apparent mineralized N in soil. The N outputs (kg N ha $^{-1}$ ) were calculated as  $NO_3^-$ -N leaching, nitrogen uptake by crop, and soil mineral N at post-harvest. The non-computed N was

calculated from the difference between N input and N output. This parameter includes the errors derived from the assumptions made and the components not determined in the N fate, such as loss of nitrogen gas.

The apparent mineralized N in soil was estimated in the control plots assuming that N input was the same as N output (Jarvis et al., 1996). Mineral N, nitrate and ammonium content in soil were determined colorimetrically in a wet soil extraction (Keeney and Nelson, 1982). The N added with irrigation was determined as the product of irrigation water nitrate concentration and the quantity of irrigation water. The N losses resulting from  $NO_3^-$ -N leaching were calculated by multiplying the drainage volume in a given period (weekly) with the nitrate concentration of the soil solution at a depth of 120 cm. The leaching of nitrate was calculated by summing the weekly leachate along the crop period. Drainage was calculated weekly at 120 cm using the water balance equation (mm):  $ET + D = (R + I) - AS$ , where ET is evapotranspiration, D is drainage below a soil depth 120 cm deeper than the root zone, R is rainfall, I is irrigation, and AS is the change in water storage to 120 cm.

The water storage of the soil profile was measured by a Diviner 2000 (Sentek) capacitance sensor. The nitrate concentration of soil solution was determined according to Keeney and Nelson (1982) on samples obtained from porous ceramic cups installed at a depth of 120 cm as recommended by Lord and Shepherd (1993). The N uptake by the crop was determined at harvest. Crop samples of 14.4 m $^2$  were taken to determine the biomass production of different parts of plants (leaves, stem, cob and grain), and the N-content (%) in each part was determined according by the Kjeldahl method (AOAC, 1990). The nitrogen fertilizer efficiency for each fertilizer treatment was calculated according to Huggins and Pan (1993) as N uptake by crop from a dose of N minus N uptake by crop in the control treatment, divided by the dose of N.

Samples of plant material (grain, leaves, stem and cob) from all treatments were oven-dried (80°C; 48 h) and milled. The  $\delta^{15}N$  and the  $\delta^{13}C$  values were determined on 1-mg sub samples (dry weight) of grain using continuous flow isotope ratio mass spectrometry. The samples were weighed, sealed into tin capsules (5 × 8 mm, Lüdi AG) and loaded into the auto sampler of an with the aid of an NC elemental analyser (NC 2500; CE Instruments, Milan, Italy). The capsule was dropped into the  $Cr_2O_3$  and  $Co_3O_4$ Ag combustion tube at 1020°C with a pulse of oxygen. The resulting oxidation products ( $CO_2$ ,  $N_xO_y$  and  $H_2O$ ) were swept into the reduction tube (Cu wire at 600°C), where nitrogen oxides were reduced to  $N_2$  and excess oxygen was removed. A magnesium perchlorate trap was used to remove the water.  $N_2$  and  $CO_2$  were separated on a GC column (Fused Silica, 0.32 mm × 0.45 mm × 27.5 m, Chrompak) at 32°C and subsequently introduced into the mass spectrometer (TermoQuest Finnigan model Delta Plus, Bremen, Germany) via a Finnigan Mat Conflo II.  $\delta$  Values (‰) were calculated using the equation:

$$\delta = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1000$$

where R is the  $^{15}\text{N}/^{14}\text{N}$  or  $^{13}\text{C}/^{12}\text{C}$  ratio.

The soil and urea  $\delta^{15}\text{N}$  were found to be 6.7 and 0.82, respectively. Grain yields were recorded for all treatments for a combination of micro-plots. Yield data were adjusted to 14% moisture.

Data were subjected to a two-way ANOVA for the effects of N fertilizer dose and irrigation system.

## Results

The grain yield for the fertilized treatments was higher in plots with sprinkler irrigation (41% and 19% higher, respectively) regardless of the nitrogen treatment (Figure 1). An increase in yield was obtained upon increasing the N dose in plots with furrow irrigation, whereas in plots with sprinkler irrigation there was a significant increase in the yield with increasing N supply in comparison to the control, but no differences between the N fertilizer rates were recorded (Table 1). The percent N content for the grain, leaves, stem and cob was higher in treatments with sprinkler irrigation regarding all organs analysed at all N fertilizer rates, with the exception of cob, for which no differences were observed in regard to any variable (Figure 2). Increasing N fertilizer rates supplied lead to a minor increase in the percent N contents in all maize plant organs studied, except for the cob, in both irrigation systems (Table 1).

The water input to system resulted from rain and irrigation. The total irrigation water applied to plots with

furrow irrigation was 1.8 time higher than in plots with sprinkler irrigation. Recorded drainage data were therefore higher in plots with furrow irrigation (around 4.3 times higher). The leaching percentage is a measure of the drainage quantity resulting from water input in the system. This parameter was 19% in sprinkler irrigation and near 51% in furrow irrigation (Table 2). The irrigation water use efficiency (IWUE) of the crop can be expressed in various ways. In our case, it is expressed as the grain yield per volume of irrigation water supplied. This method of calculating the IWUE gives an agricultural and environmental view of the amount of water used in each irrigation system to obtain the final product. The maize grain yield per cubic metre of water supplied is higher in

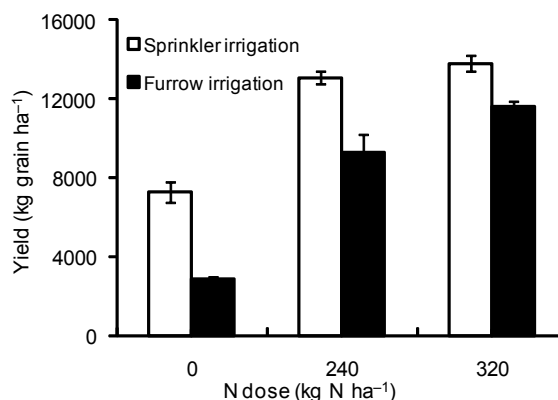


Figure 1 – Yield of maize crop fertilized with 0, 240 and 320 kg N ha<sup>-1</sup> and two types of irrigation, sprinkler and furrow. Values are means  $\pm$  SE.

Table 1 – Probability levels of ANOVA on the effects of N fertilizer rate and irrigation system and their interaction on determined parameters.

	N fertilizer	Irrigation	N fertilizer $\times$ Irrigation
Yield	0.000	0.000	0.086
Grain %N	0.000	0.000	0.002
Stem %N	0.003	0.002	0.027
Leaves %N	0.014	0.001	0.332
Cob %N	0.118	0.078	0.702
IWUE	0.000	0.000	0.020
N uptake	0.000	0.000	0.008
NUE	0.000	0.000	0.008
NO <sub>3</sub> <sup>-</sup> -N leaching	0.000	0.000	0.005
Grain $\delta\text{C}$	0.277	0.272	0.398
Stem $\delta\text{C}$	0.265	0.006	0.390
Leaves $\delta\text{C}$	0.011	0.000	0.023
Cob $\delta\text{C}$	0.006	0.000	0.005
Grain $\delta\text{N}$	0.231	0.000	0.000
Stem $\delta\text{N}$	0.293	0.000	0.000
Leaves $\delta\text{N}$	0.000	0.000	0.000
Cob $\delta\text{N}$	0.060	0.081	0.036

sprinkler irrigation than in furrow irrigation, regardless of the N dose. For the N doses investigated (240 and 320 kg N ha<sup>-1</sup>), the yield with sprinkler irrigation is more than 2 kg of grain per cubic metre of water supplied, whereas for furrow irrigation it is approximately 1 kg (Table 3).

The N inputs taken into account in the system were soil mineral N at pre-sowing, N added at side dress fertilization, N added with irrigation water and apparent

mineralized N in soil. The mineral N content of plot's soil ( $\text{NO}_3^- + \text{NH}_4^+$ ) under sprinkler irrigation was slightly higher than the N content in plots under furrow irrigation at sowing. The N added by the irrigation water was 1.7 time higher in plots under furrow irrigation (the N concentration in irrigation water was the same but the volume of water supplied by furrow irrigation was larger). The estimated amount of N mineralized

Table 2 – Water parameters, cumulative rain, irrigation and drainage and leaching in the two types of irrigation, sprinkler and furrow. Values are means  $\pm$  SE.

	Cumulative rain	Cumulative irrigation	Cumulative drainage	Leaching percentage
	L m <sup>-2</sup>			%
Sprinkler irrigation	156	600	144 $\pm$ 6	19
Furrow irrigation	156	1082	626 $\pm$ 3	51

Table 3 – Irrigation water use efficiency to maize fertilized with 0, 240 and 320 kg N ha<sup>-1</sup> and the two types of irrigation, sprinkler and furrow. Values are means  $\pm$  SE.

	N dose (kg N ha <sup>-1</sup> )		
	0	240	320
	kg grain m <sup>-3</sup> applied irrigation water		
Sprinkler irrigation	1.2 $\pm$ 0.1	2.2 $\pm$ 0.1	2.3 $\pm$ 0.1
Furrow irrigation	0.3 $\pm$ 0.0	0.9 $\pm$ 0.1	1.1 $\pm$ 0.0

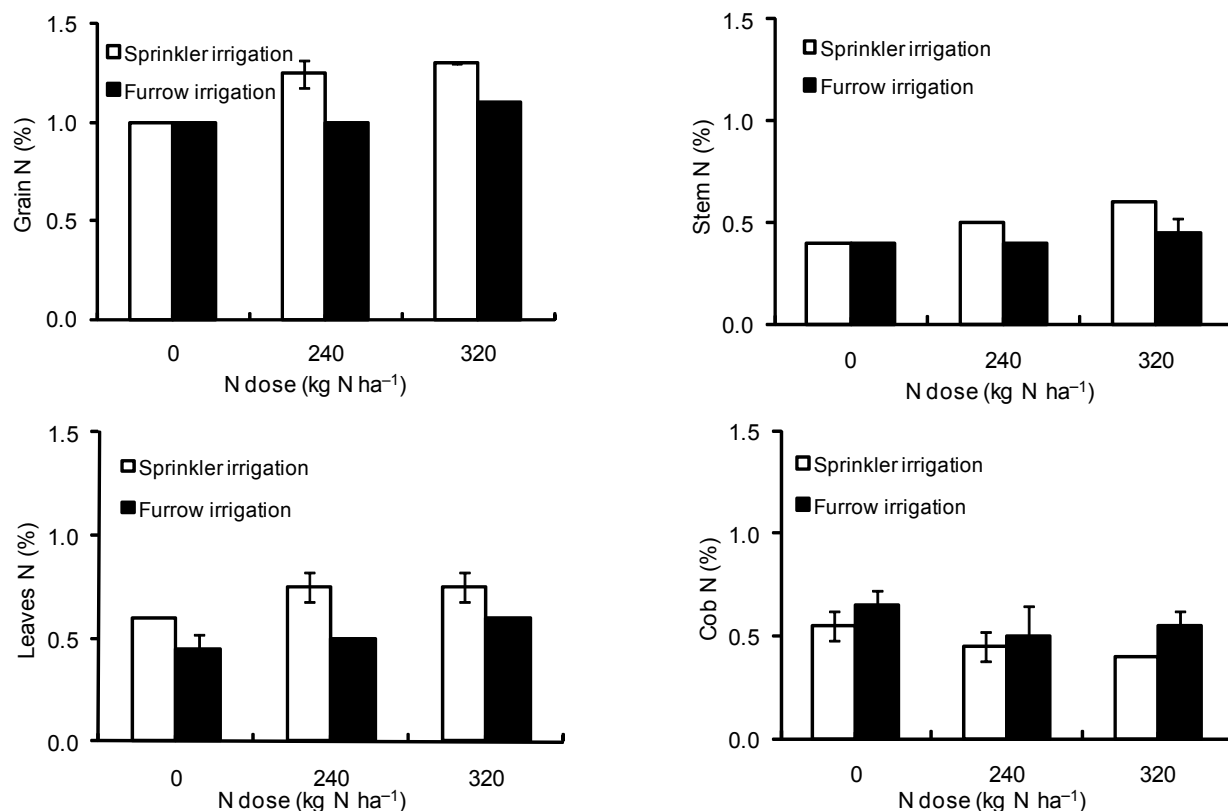


Figure 2 – Organic nitrogen content in parts of maize crop fertilized with N under types of irrigation: sprinkler and furrow. Values are means  $\pm$  SE.

during the crop period was three times higher in plots under sprinkler irrigation (Tables 4 and 5). The N outputs were  $\text{NO}_3^-$ -N leaching, N uptake by the crop, and post-harvest soil mineral N. The N loss resulting from  $\text{NO}_3^-$ -N leaching was significantly higher in plots with furrow irrigation and increased with increasing N rates for both types of irrigation (Tables 4 and 5). The percent  $\text{NO}_3^-$ -N leaching as related to N available to the crop (N mineral presowing + N fertilizer) was, respectively, 13%, 11% and 11% for the control, 240 and 320 kg N ha<sup>-1</sup> treatments under sprinkler irrigation, and 37%, 17% and 19% under furrow irrigation. The N uptake by the crop followed the same trend as the grain yield; it was higher with sprinkler irrigation regardless of the added N fertilizer rate. N uptake by the crop had already reached saturation at a rate of 240 kg N ha<sup>-1</sup> under sprinkler irrigation, whereas no saturation was observed in furrow irrigation at neither of the studied N rates. The mineral N content in soil at harvest increased with increasing N rates in both irrigation systems, although this

parameter tended to be higher in plots under sprinkler irrigation (Table 1).

The non-computed N, estimated from the difference between the N input and output, was highly variable – 54 to 104 kg N ha<sup>-1</sup> in plots with N-fertilization – and did not significantly differ between treatments. Nitrogen fertilizer use efficiency (NUE) ratios, estimated from the N fate data, demonstrated that 60% of the supplied N was taken up by the crop in sprinkler irrigation, whereas under furrow irrigation and at N rates of 240 and 320 kg N ha<sup>-1</sup>, only 30% and 40% N were taken up by the crop, respectively.

The  $\delta^{13}\text{C}$  values of all plant organs, especially stems and leaves, were lower (more negative) for almost all treatments under furrow irrigation (Figure 3). However, whereas  $\delta^{13}\text{C}$  for the plant organs was not affected by N rate supplied under furrow irrigation, values for sprinkler irrigation system were lower (more negative) for the rate of 320 kg N ha<sup>-1</sup> in comparison to control; values for 240 kg N ha<sup>-1</sup> did not significantly differ from that of other

Table 4 – N fate in maize crop fertilized with 0, 240 and 320 kg N ha<sup>-1</sup> with sprinkler irrigation. Values are means  $\pm$  SE.

Sprinkler irrigation	kg N ha <sup>-1</sup>		
N dose	0 kg N ha <sup>-1</sup>	240 kg N ha <sup>-1</sup>	320 kg N ha <sup>-1</sup>
Soil mineral N at presowing	63 $\pm$ 3	86 $\pm$ 5	97 $\pm$ 10
N fertilizer	0	240	320
N of irrigation	19	19	19
Apparent mineralized N	83 $\pm$ 10	83 $\pm$ 10	83 $\pm$ 10
N INPUTS	165 $\pm$ 10	428 $\pm$ 11	519 $\pm$ 14
$\text{NO}_3^-$ -N Leaching	8 $\pm$ 1	37 $\pm$ 2	46 $\pm$ 1
Uptake N by crop	104 $\pm$ 8	254 $\pm$ 14	266 $\pm$ 11
Soil mineral N at postharvest	53 $\pm$ 4	84 $\pm$ 32	103 $\pm$ 56
N OUTPUTS	165 $\pm$ 8	374 $\pm$ 34	415 $\pm$ 57
No computed N	0 $\pm$ 13	54 $\pm$ 36	104 $\pm$ 59
N-fertilizer use efficiency (%)		0.6 $\pm$ 0.1	0.6 $\pm$ 0.0

Table 5 – N fate in maize crop fertilized with 0, 240 and 320 kg N ha<sup>-1</sup> with furrow irrigation. Values are means  $\pm$  SE.

Furrow irrigation	kg N ha <sup>-1</sup>		
N dose	0 kg N ha <sup>-1</sup>	240 kg N ha <sup>-1</sup>	320 kg N ha <sup>-1</sup>
Soil mineral N at presowing	56 $\pm$ 1	69 $\pm$ 9	63 $\pm$ 6
N fertilizer	0	240	320
N of irrigation	33	33	33
Apparent mineralized N	28 $\pm$ 1	28 $\pm$ 1	28 $\pm$ 1
N INPUTS	117 $\pm$ (1)	370 $\pm$ (9)	444 $\pm$ (6)
$\text{NO}_3^-$ -N Leaching	21 $\pm$ 1	52 $\pm$ 1	74 $\pm$ 2
Uptake N by crop	53 $\pm$ 1	128 $\pm$ 18	192 $\pm$ 19
Soil mineral N at postharvest	44 $\pm$ 2	55 $\pm$ 3	66 $\pm$ 1
N OUTPUTS	117 $\pm$ 2	234 $\pm$ 18	332 $\pm$ 17
No computed N	0 $\pm$ 2	136 $\pm$ 18	112 $\pm$ 17
N-fertilizer use efficiency (%)		0.3 $\pm$ 0.1	0.4 $\pm$ 0.1

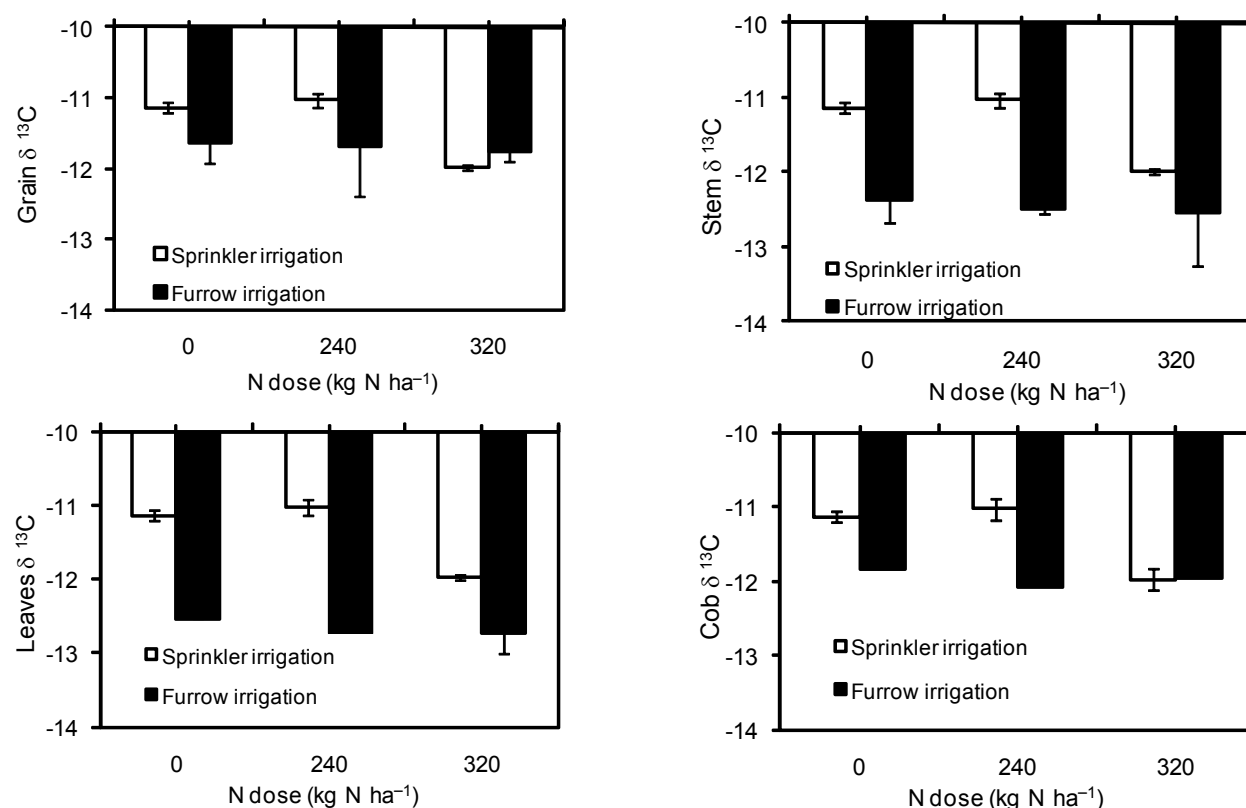


Figure 3 – Natural abundance of  $^{13}\text{C}$  ( $\delta^{13}\text{C}$ ) in parts of maize crop fertilized with N under types of irrigation: sprinkler and furrow. Values are means  $\pm$  SE.

treatments (Table 1). The  $\delta^{15}\text{N}$  values for grain, stem, leaves and cob decrease with increasing N fertilization rates in plots under sprinkler irrigation, although all values always remained positive (Figure 4). The trend in plots under furrow irrigation was the opposite, since  $\delta^{15}\text{N}$  increased in all plant organs analysed, except cob, with increasing N-dose supplied (Table 1). The weighted average of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the whole plant follows the same trend of data obtained for grain (data not shown).

## Discussion

The low efficiency of furrow irrigation has already been reported (Al-Jamal et al., 2000). Irrigation is most efficient when the added water volume nears the crop evapotranspiration rate, thereby minimising leaching and drainage. In studies in which response to N fertilization has been evaluated under varying irrigation systems, authors such as Román et al. (1996) have estimated that drainage of about 20% of the irrigation water supplied is sufficient to estimate the effect of different N-fertilizer treatments. This requirement was met in this study since the minimum leaching flow in sprinkler irrigation neared 20%.

Application of N-fertilizer increased the irrigation water use efficiency (IWUE) by circa 1.9 and 3.3 times in sprinkler and furrow irrigation, respectively, since the main limiting factor in the control treatment was the availability of nitrogen. The IWUE was higher in sprin-

kler irrigation than in furrow irrigation because the yield in furrow irrigation was lower, and the volume of water supplied was higher. Several authors have shown that IWUE values for several crops, such as cotton (*Gossypium hirsutum*) (Dagdelen et al., 2006), onion (*Allium cepa*) (Al-Jamal et al., 2000), potato (*Solanum tuberosum*) and maize (Yuan et al., 2003), decrease with increasing irrigation water volume. Sprinkler irrigation not only reduces the amount of water supplied to the crop comparatively to furrow irrigation, but also the water is provided in a more fractionated way, leading to a lower reduction of maximum soil moisture. Drainage can also be reduced and water use efficiency increased by adjusting the irrigation intensity to each instance it is needed (Román et al., 1999).

Diez et al. (1997) have shown that in a maize crop subject traditional (furrow) vs. efficient irrigation, the ratio of drainage of both types was similar to that herein reported. However, these authors reported lower quantities of drainage and higher ratios of  $\text{NO}_3^-$ -N leaching between traditional and efficient irrigation (ratio = 5) than that reported in this study (ratio = 1.4 – 2.6). Pang and Letey (1998) added amounts of water and nitrogen fertilizers to maize crops and demonstrated that larger amounts of irrigation water, which led to larger amounts of deep percolation, resulted in far more  $\text{NO}_3^-$ -N leaching and lower yields. Similar results were recorded in this study, namely that furrow irrigation uses larger quantity of water and leads to important N losses as a result

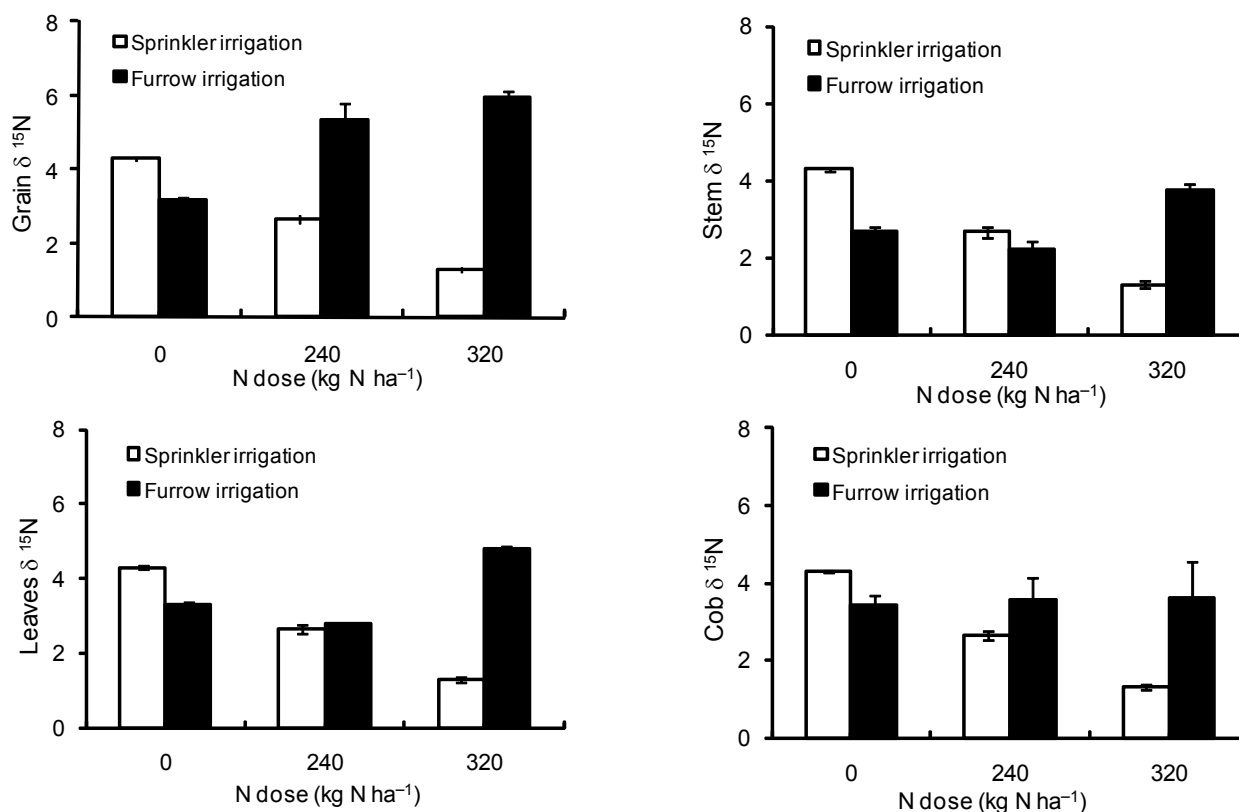


Figure 4 – Natural abundance of  $^{15}\text{N}$  ( $\delta^{15}\text{N}$ ) in parts of maize crop fertilized with N under types of irrigation: sprinkler and furrow. Values are means  $\pm$  SE.

of  $\text{NO}_3^-$ -N leaching, and also, probably, denitrification. Denitrification is higher in the anaerobic soil environment as a result of flooding; furrow irrigation involves flooding the plot for two to three days so this irrigation technique probably leads to significant N losses.

The higher N loss resulting from leaching and denitrification that occurs in a furrow irrigation system in comparison to sprinkler irrigation results in lower N availability for the crop and, therefore, to lower N absorption. This difference in N availability could have caused the differences in maize yield registered for both types of irrigation. Nitrogen and water are both key factors for high yields, especially in crops such as maize. Yields recorded in this study also show that the ratio of N necessary for optimum yield is lower under sprinkler irrigation (240 kg N ha<sup>-1</sup>) than under furrow irrigation (320 kg N ha<sup>-1</sup>), which affect differently the N cycle. Similar conclusions, i.e. increased leaching and runoff of available N occurs in a furrow irrigation system in comparison to sprinkler irrigation, and that sprinkler-irrigated crops, such as sugar beets, probably need less applied N than furrow-irrigated crops because of reduced N losses resulting from leaching and runoff, are commonly found in the specialized literature (Eckhoff et al., 2005).

The NUE, expressed as N uptake by the crop at a certain N dose minus the N uptake by the control divided by ratio of N supplied by the fertilizer, can be calculated from the N fate data. In plots under sprinkler irrigation, NUE decreased with increasing N ratio; O'Neill et al.

(2004) and Di Paolo and Rinaldi (2008) also reported similar findings. However, similar effect was not observed in plots under furrow irrigation. In those plots, maize yield increased with increasing N ratio, although this was accompanied by lower NUE resulting from increased  $\text{NO}_3^-$ -N leaching, and probably increased gaseous emissions. O'Neill et al. (2004) and Di Paolo and Rinaldi (2008) have also reported higher NUEs when more water was available in the soil but in water-deficient irrigation conditions, where water was the main limiting factor.

In general, the  $\delta^{13}\text{C}$  value was lower (more negative) under furrow irrigation for all plant organs analyzed and N fertilization ratios. Changes in C isotope discrimination by plants can result from temporary change in the growth environment, especially in regard to  $\text{C}_3$  plants, which have higher variation of the C isotope composition than  $\text{C}_4$  plants (Farquhar et al., 1989). Increased salinity (Guy and Reid, 1986), decreased soil water availability (Farquhar and Richards, 1984), soil compaction (Masle and Farquhar, 1988) and increased vapour-pressure deficit (Winter et al., 1982) also result in lower values of  $^{13}\text{C}$  discrimination (less-negative  $\delta^{13}\text{C}$  values). Effects such as soil compaction resulting from increased mass of water stood by the soil after each irrigation cycle, can be observed in soils under furrow irrigation. Plots under furrow irrigation can also stand greater vapour pressure deficit than plots under sprinkler irrigation for longer periods as a result of the lower irrigation frequency (weekly for furrow irrigation and every

two days for sprinkler irrigation). As a matter of fact, cracked soil was observed in plots under furrow irrigation in the days preceding an irrigation cycle.

Working in greenhouse conditions, Dercon et al. (2006) reported that a high vapour pressure deficit may be a reason for limited opening of stomata, even in an optimal water environment. Dercon et al. (2006) also found that temporary hydric stress of  $C_4$  plants such as maize, affects the isotopic composition of the plant, conversely to that observed for  $C_3$  plants, that is, a temporary hydric stress elicits more negative  $\delta^{13}C$  in these plants. This effect has been primarily attributed to decreased stomatal conductance and changes in photosynthetic capacity per unit leaf area, which may affect the C isotope composition (Masle and Farquhar, 1988).

Nitrogen ratios did not influence the value of  $\delta^{13}C$  of maize plants under furrow irrigation, whereas the highest N ratio brought out more negative  $\delta^{13}C$  values on maize plants under sprinkler irrigation. Levels of nitrogen nutrition have small and variable effects on the isotopic discrimination of  $^{13}C$  in  $C_3$  species such as wheat (Condon et al., 1992), and the N ratio does not affect the  $\delta^{13}C$  value of leaves in some  $C_4$  plant, such as *Amaranthus cruentus* (Tazoe et al., 2006). In contrast, Meinzer and Zhu (1998) reported that N-stress reduces  $\delta^{13}C$  in  $C_4$  species because of a reduction of the partitioning of N to Rubisco relative to phosphoenolpyruvate carboxylase [PEPC] can increase the  $CO_2$  leakiness of the bundle sheath. However, contrasting patterns of N partitioning between Rubisco and PEPC have been reported in  $C_4$  species (Sage et al., 1987). However, all of these studies were performed in greenhouse or a growth chamber under hydroponic conditions; therefore it is possible that these varying growth conditions could affect the results obtained concerning the effect of N ratio on the C isotope composition of plants.

Kinetic isotope fractionation during biological and physical N transformations generally results in  $^{15}N$  enrichment of the substrate, since molecules bearing lighter isotopes tend to react faster than those bearing heavier isotopes (Criss, 1999). Conditions intrinsic to furrow irrigation, such as the large volume of water added to soil receives and a two-to-three days a week cycle, keep the soil under anaerobic conditions during the irrigation period. In such conditions, the rate of  $NO_3^-$ -N leaching is higher than under sprinkler irrigation as shown, and so is the rate of denitrification, a well-known process in the N cycle of flooded soils (Thompson, 1996). Soils with a high level of denitrification will have higher  $^{15}N$  contents (Högberg and Johansson, 1993). Moreover, when the optimum conditions for denitrification occur, the rate of denitrification increases when the availability of N increases in the soil (Mosier et al., 1982), which would explain why the  $\delta^{15}N$  of plants cultivated under furrow irrigation increases with increasing N fertilization ratio.

The contribution of N fertilizer to the plant nitrogen content (NUE) is higher under sprinkler irrigation. Therefore the  $\delta^{15}N$  of plants cultivated under sprinkler

irrigation should become closer to that of the N fertilizer with the increasing the N ratio, seeing that  $\delta^{15}N$  for urea is close to zero (0.82) and average  $\delta^{15}N$  for soil is 6.7. Serret et al. (2008) have reported that  $\delta^{15}N$  for a wheat (*Triticum aestivum*) crop under well-irrigated field conditions decreases with increasing addition of urea. The lack of linearity in the change of wheat grain  $\delta^{15}N$  with increasing amount of chemical fertiliser is an indication that factors such as volatilization, denitrification, and nitrification followed by denitrification or leaching, play a role in determining plant  $\delta^{15}N$  in addition to the signature of the N source (Serret et al., 2008). On the other hand, the soil moisture profile arising from furrow irrigation is deeper and has higher water content at depth than with sprinkler irrigation (data not shown). This water distribution probably causes a difference in the root structure of plants grown under these two irrigation systems, that is, the roots in plots under furrow irrigation grow deeper than in those under sprinkler irrigation. Various authors have described  $^{15}N$  enrichment of soil mineral N with depth (Steele et al., 1981; Tiessen et al., 1984), therefore the percentage of  $^{15}N$  available to plant roots under furrow irrigation might be higher than for plant roots under sprinkler irrigation.

Maize yield with sprinkler irrigation is higher than with furrow irrigation, regardless of the applied dose of nitrogen, due to a higher availability of N to the crop. The IWUE and NUE are higher in plants cultivated with sprinkler irrigation than with furrow irrigation, which means that sprinkler irrigation is a more water-efficient irrigation system which requires less nitrogen fertilization to provide an optimum yield and therefore has a lower environmental impact due to nitrate leaching.

In conclusion, the N dose effect in the two types of irrigation on  $\delta^{15}N$  can be explained by the N cycle processes which occur with each type of irrigation. N losses due to  $NO_3^-$ -N leaching and denitrification prevail in furrow irrigation, whereas a more efficient use of N fertilizer prevails in sprinkler irrigation. The lower  $\delta^{13}C$  found in various organs of the maize plant grown with furrow irrigation suggests temporary hydric deficits occur with this type of irrigation. Only limited effects of N dose on  $\delta^{13}C$  have been observed.

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